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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY SEVERE INJURY

ANNUAL PROGRESS REPORT

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FEBRUARY 1, 1989

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-86-C-6097

University of Massachusetts
Worcester, Massachusetts 01605

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of Massachusetts; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Worcester, MA 01605	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-86-C-6097
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62772A	3S16 2772A874 ✓	AD	127

11. TITLE (Include Security Classification)

Studies of Altered Response to Infection Induced by Severe Injury

12. PERSONAL AUTHOR(S)

Carol L. Miller-Graziano, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 1/24/88 TO 1/24/89	1989 February 1	28

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	RA II; Monoclonals; Recombinant DNA; Thermal Injury; Infection; Antibody; Leukocytes; Guinea Pigs
06	13		
06	05		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This research's main goals are to develop assays to monitor the appearance and progression of post-trauma immunosuppression and to examine prophylactic modalities which may reverse or reduce this immunosuppression. This contract year we have successfully adapted assays for IL-1, IL-6, and Transforming Growth Factor$_\beta$(TGF$_\beta$) to use with patient monocytes (MØ). We have demonstrated that MØ levels of IL-6 and TGF$_\beta$ are elevated in immunosuppressed patients probably accounting for their increased hepatocyte dysfunction(IL-6) and maintenance of their immunosuppression(TGF$_\beta$). Elevation of patients MØ's production of tumor necrosis factor(TNF) was shown to occur primarily in the cell-associated form of TNF and this elevated TNF occurs concomitant to an elevation in MØ PGE$_2$. Prophylactic treatment with IL-4 was shown to downregulate both TNF and PGE$_2$ while treatment with indomethacin decreased PGE$_2$ but caused increased TNF release. Our data indicated that post-trauma increases in the number of MØ expressing the 72KD receptor for IgG(FcRI) is correlated to the immunosuppressed patients' MØ increased production of IL-6, TNF, and decreased IL-1 production. Consequently, monitoring the shift in the ratio of patients' FcRI$^+$ to FcRI$^-$ MØ may allow detection of post-trauma immune changes without difficult bioassay performance.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Virginia M. Miller	301/663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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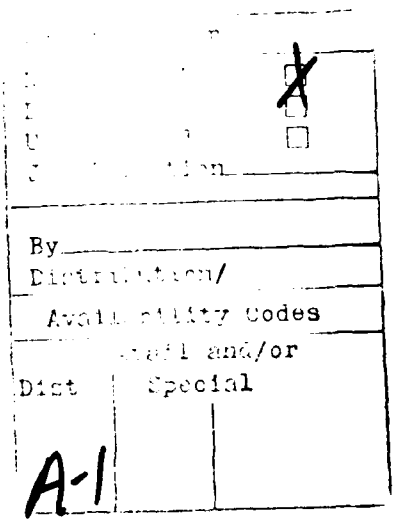

TABLE OF CONTENTS

INTRODUCTION

The main goals of this research are: 1) to develop assays which can be used to moniter the development and progression of immunosuppression in post-trauma patients and (2) to investigate possible prophylactic modalities which can be specifically targeted to reversing or reducing post-trauma immune aberrations. To achieve these goals, we must characterize what monocyte (MØ) and/or T-cell functions are aberrant after a severe injury and define how these aberrant MØ and/or T-cell functions mediate post-trauma immunosuppression and metabolic aberrations. Our focus over the entire contract period has been primarily on MØ and their products as well as T-cell lymphokine effects on these products. This laboratory and others have clearly demonstrated that excessive MØ production of PGE_2, concomitant to depression of other MØ T-cell interactive functions, plays a major role in the development of post-trauma immunosuppression. We have also shown that a post-trauma shift in the relative proportions of MØ toward large numbers of a more immunosuppressive MØ subset and fewer numbers of the immunostimulatory MØ subset can be linked to the development of many MØ aberrations. We have defined these MØ subsets on the basis of their expression of the FcRI receptor for IgG. The $FcRI^+$ MØ subset can be segregated on the basis of its rosetting with anti Rh coated human erythrocytes. During this contract year, we have continued to pursue our goals. We have demonstrated that trauma patients' MØ antigen presentation capacity is depressed compared to that of normal controls. We have further defined the consequences of increased numbers of $FcRI^+$ and decreased numbers of the $FcRI^-$ MØ subset, showing that the $FcRI^-$ MØ subset has the greater antigen presentation capacity (APC).
These data indicating a post-trauma shift in MØ subsets may account for some of the depressed MØ APC seen in the immunosuppressed trauma patients. However, the post-trauma MØ antigen presenting defect has also been demonstrated to occur in immunosuppressed patients' $FcRI^-$ MØ subset. This result implies that the shift in MØ subset does not totally account for post-trauma immunosuppression.

We have also made significant progress in assessing new bioassays for monitoring patients MØ aberrations by initiating measurement of patients' MØ IL-1, IL-6 and TGF_β production. Interleukin 1 (IL-1) is a monokine produced in membrane bound and secreted form. IL-1 has several similar effects to TNFα, it is an endogeneous pyrogen and it has also been suggested to be necessary for proliferation of certain types of T-cells. MØ IL-1 is reportedly upregulated or downregulated post-injury, depending on the assay systems in which IL-1 activity was measured. Therefore, we decided to measure IL-1 activity of MØ in the IL-1 specific D10.G4.1 bioassay. In contrast to the depression of IL-1, IL-6 is another monokine reportedly elevated in the serum of post-injury patients. We can now assess MØ as the source of the elevated serum IL-6 levels in post-trauma patients and determine if elevated IL-6 contributes to the trauma in patients' metabolic dysfunction. IL-6 is a potent inducer of acute phase reactants. TGF_β is another mediator produced by MØ as well as by other cell

types. TGF_β is a known inhibitor of T-cell proliferation and other immune functions. However, TGF_β augments proliferation of connective tissue cells helping wound healing. Therefore, elevated TGF_β levels produced by post-injury MØ might have both positive and negative effects depending on the localization of the TGF_β producing MØ. Systemic TGF_β production would be predominantly immunosuppressive. We are assessing the in vitro TGF_β production by patients' circulating monocytes post-injury.

In addition to these newly established assays for monokine measurement, we have successfully continued our analysis of TNF production by trauma and burn patients' MØ. Tumor necrosis factor is linked to mediating septic shock and death post-injury. Besides demonstrating moderately elevated secreted MØ TNF levels, we have also shown tremendous elevation in the trauma patients' MØ cell-associated TNF, as measured in the sonicated MØ lysates in our L-M cell assay. Third, we have confirmed our findings in the previous contract year, that the $FcRI^+$ MØ subset produces the majority of PGE_2, as well as the majority of the secreted and cell-associated TNF in the patients and in the normals. This data further increases the importance of our previous observation, that the post-trauma patients have increased numbers of the FcRI positive MØ subset. Therefore, by examining patients' MØ subset ratios, we can associate a change in MØ functions with a change in MØ phenotype. Consequently, the increased appearance of the $FcRI^+$MØ subset could quickly detect the onset of immunoincompetence in trauma patients, and could help to identify and segregate combat victims requiring more intensive care. Fourth, we pursued our goal of testing new prophylactic therapies. In this contract period, we have extensively studied the effect of Indomethacin and Interleukin 4 (IL-4) on the post-injury MØ functions. Indomethacin like Ibuprofen, is a cyclo-oxygenese inhibitor with an inhibitory effect on MØ PGE_2 production. Therefore, utilization of Indomethacin to downregulate the extremely elevated post-trauma MØ PGE_2 production seems to be a reasonable prophylactic approach. However, our experiments demonstrated that Indomethacin treatment, while downregulating patients' MØ PGE_2 levels, concomitantly further enhanced the already elevated patient MØ TNF levels. Furthermore, Indomethacin induced the secretion of the massively elevated cell-associated TNF present in the patients' MØ. Since circulating TNF has been shown to mediate septic shock, Indomethacin induced release of massive amounts of TNF could have important clinical relevance in the treatment of immunocompromised patients. In this year, we have also demonstrated a novel effect of IL-4 on cyclo-oxygenase products in post-injury and normal patients' MØ. IL-4 downregulated MØ PGE_2 and TNF production concomitantly. IL-4 also appears to prevent the secretion of the highly elevated cell-associated MØ TNF in the post-trauma patients. In summary, in this year, we have made significant advances in pursuing our goals. We have initiated new methods to monitor immunosuppression developed in severely injured patients, and we have demonstrated that immunosuppressed patients have extremely elevated levels of cell-associated TNF. We have investigated the prophylatic potential of Indomethacin and found that in vitro treatment with Indomethacin can further enhance the TNF production by the immunocompromised patients' MØ. In striking contrast, IL-4 treatment can downregulate the patients' elevated MØ TNF levels suggesting that IL-4 may have prophylactic potential.

METHODS

MØ plasminogen activator (PA), procoagulant activity (PCA) and lysozyme assays are performed as previously described. MØ antigen presentation capacity was tested as described by Goeken et al. Briefly, selective adherence separated patients' and normals' MØ and FcRI MØ subsets were pulsed overnight with 5 Lf/ml tetanus toxoid antigen. After overnight culture excess antigen was removed by extensive washing and MØ were recombined with syngeneic T-cells at a 1:6 ratio. T-cell proliferation was measured on the 6th day of incubation with 18 hours ^{3}H-Td-R incorporation. MØ PGE_2 is measured in the ELISA assay, which was adopted in our laboratory during the previous contract year. MØ TNF levels were determined in the cell-free MØ supernates (secreted TNF) and in the sonicated MØ lysates (cell-associated TNF) using the L-M cell bioassay as previously described (1). Secreted IL-1 activity was measured in MØ supernates. The IL-1 specific D10.G4.1 cells were cultured in the presence of different dilutions of the IL-1 containing MØ supernates. 2.5 μg/ml Con A was used as a co-mitogen in RPMI media containing $5x10^{-5}$ M 2ME and 5% FBS. The proliferation of $2x10^{4}$ D10.G4.1 cells/well was measured using a ^{3}H-Thymidine pulse for the last 18 hours of the 72 hours proliferation assay. Sample activity was calculated by comparing the dilution of the samples to the dilution of the IL-1 standard resulting in 50% maximal proliferation. MØ IL-6 production was measured in the MØ supernates as previously described, using the B9 hybridoma cell line, which is highly specific for IL-6 as previously described (2). Briefly, $2x10^{4}$ B9 cells were seeded in serial dilutions of the MØ supernate samples or IL-6 standard on a 96 well plate. B9 proliferation was measured by 3H-TdR incorporation during the last 16 hours of the 96 hours proliferation assay. Activity of the samples was calculated by a computer program using our Compaq computer, using the following formula:

$$\text{Sample activity} = \text{Stand. activity} \times 2^{\text{Sample EP-St.EP}}$$

The Mv1Lu (mink lung) cell assay was utilized to measure the TGF_β production by trauma patients' and normal monocytes. Proliferation of the Mv1Lu cells is inhibited by TGF_β. TGF_β activity produced by MØ is in a latent form in the MØ supernates, because active TGF_β is complexed with a TGF_β binding protein, which is biologically inactive. TGF_β can be converted to its free, biologically active form with acid treatment. Therefore, our MØ supernate samples are acid treated (pH 2.5-2.8) for 2 hours. Their pH is then adjusted back to pH 7.32, filtered, and used for TGF_β determination in the Mv1Lu bioassay. Activity of the MØ supernates is calculated for the dilution of the sample and the standard resulting in half maximal inhibitors by the following formula:

$$\text{Sample activity: Std. Activity} \times 2^{\text{Sample EP-St. EP}}$$

MØ are isolated from each patient as early as 1-3 days post-injury, and blood is collected biweekly during their hospitalization. Each patient's blood sample is processed along with a normal's control blood donated by the research and hospital staff at the UMMC. MØ were stimulated with 20 ug/ml muramyldipeptide (MDP) alone, with a combination of 10^{-6} M Indomethacin (INDO) + MDP or with a suboptimal dose of Interferon gamma (IFN$_\gamma$) (20 mg/ml) plus 20 ug/ml MDP. MØ supernates were collected after 16-20 hours incubation and the adherent MØ were collected with short EDTA treatment followed by scraping. MØ were used for cell-associated TNF and PCA determinations after freezing-thawing and sonication. MØ subsets are separated on the basis of the density of their high affinity Fc receptors for human IgG_1 and IgG_3 (FcRI) by rosetting the MØ

with anti Rh-coated erythrocytes. Subset experiments are performed on trauma and burn patients to delineate any differential monokine producing capacity or any differential stimulation requirements for the FcRI MØ subset. The cyclo-oxygenase inhibitor, Indomethacin (Indo) was used to inhibit MØ PGE_2 production. The effect of stimulation with Indo in combination with MDP was studied for MØ TNF induction. IL-4 alone or in combination with Indo + MDP was also utilized for MØ stimulation. The levels of MØ monokine production may vary from individual to individual, with some high and some low responders. Consequently, Wilcoxon Signed Rank and Mann-Whitney U nonparametric statistics were utilized when appropriate to compare changes in MØ responses after IFN_γ and MDP stimulation.

RESULTS AND DISCUSSION

In the period covered by the third year of contract No. DAMD 17-86-C-6091, several goals have been attained. Thirty-six patients have been monitored this year, including 19 trauma and 17 burn patients. Of these patients, eleven were studied in the first three months, nine patients during the second four months and sixteen patients during the last five months of the year. Of 36 trauma patients, 6 succumbed to fatal sepsis. Out of the total 36 patients studied, Table 1 summarizes the abnormal MØ functions of the 22 immunocompromised patients who also experienced septic episodes (9 trauma and 13 burn). The patients' mononuclear cell proliferation to PHA was depressed from 42-93 percent. The plasminogen activator production by these patients' MØ was also decreased in all of the immunocompromised patients. Decreased MØ PA and PHA induced T-cell proliferation was concomitant to increased MØ PGE_2 production and poor prognosis. These data confirm our previous observations that decreased MØ PA and PHA induced T-cell proliferation occurs with concomitant elevations in MØ PGE_2 levels in trauma and burn patients who experienced septic complications. Immunocompetent patients, who had no septic episodes, also had no dramatic decrease in their PA and PHA response. However, these patients' MØ PGE_2 levels were moderately elevated.

Antigen presentation capacity (APC) of post-injury patients' MØ was assessed in some experiments. We found a decreased APC capacity of patients' MØ (Table 2), as well as their $FcRI^+$ and $FcRI^-$MØ subsets. The $FcRI^-$MØ subset has been previously shown as the main antigen presenting MØ in normals. Our present data confirmed again, that in the presence of antigen pulsed $FcRI^-$MØ, T-cell proliferation was significantly higher than that of the $FcRI^+$ MØ. In the post-trauma patients, however, antigen presentation capacity of the $FcRI^-$MØ was significantly depressed. T-cell proliferation in the presence of the low APC $FcRI^+$ MØ also decreased in the immunocompromised trauma patients. These data suggest that in immunocompromised post-injury patients, the antigen presenting function of the MØ is depressed. However, the depressed APC does not solely result from the MØ subset switch toward fewer $FcRI^-$ antigen presenting MØ. Both the depressed APC of the patients' $FcRI^-$ MØ as well as their fewer numbers of the antigen presenting $FcRI^-$MØ may contribute to these patients higher risk of infections. The lower APC, exhibited by the $FcRI^+$ MØ subset, could be explained by their excessive PGE_2 production and consequent inhibition of T-cell proliferation. The $FcRI^+$ MØ produce the majority of MØ PGE_2. Therefore, inhibitors of high PGE_2 levels in the $FcRI^+$ MØ should increase the APC of the

$FcRI^+$ MØ. As demonstrated in Table 3, T-cell proliferation was still much lower when $FcRI^+$ MØ were the antigen presenting cells, even in the presence of the cyclo-oxygenase inhibitor, Indomethacin. These data suggest that the inability of $FcRI^+$ MØ to act as antigen presenting cells, is not primarily due to their excessive production of PGE_2. Other mediators such as (IL-1, TGF_β) either produced or not produced by the $FcRI^+$ MØ subset might also be responsible for the lower APC of the $FcRI^+$ MØ subset. Alternately, the $FcRI^+$ MØ subset might have differential APC capacity due to its antigen processing capacity. Because it processes or presents antigen less efficiently, IL-1 produced by MØ, also affects T-cell development and proliferation. We have established the DIO.G4.1 bioassay for IL-1 determination in our laboratory during this contract year. During the first quarter of this contract year, data from the literature suggested, that the D10.G4.1 murine helper T-cells might also be sensitive to IL-6 as well as IL-1. Therefore, any contaminating IL-6 in our MØ supernates samples could mimic IL-1 activity. More recent papers, however, confirmed that the D10-G4.1 assay is specific only for IL-1 and not affected by IL-6. Table 4 shows data indicating decreased IL-1 production in immunocompromised trauma patients' MØ compared to a normal control. IL-1 production of the patients' MØ decreased 5 days after injury compared to the same patients' level 2 days post-injury or to the normals. We have also assesed the IL-1 production by the patients' FcRI MØ subsets. The decrease in patients' MØ Il-1 production appeared primarily in the $FcRI^+$ MØ subset. Since these post-injury immunocompromised patients experience higher proportions of the $FcRI^+$ MØ than the normals, the decreased IL-1 production in the $FcRI^+$ MØ subset might result in the IL-1 decrease observed in the whole MØ population. The D10.G4.1 bioassay measures both $IL\text{-}1_\alpha$ and $IL\text{-}1_\beta$ activities. However, patients' MØ might produce $IL\text{-}1_\alpha$ and/or $IL\text{-}1_\beta$ differently from normals. Consequently, we have also initiated isoelectric focusing and immobiline gel-separation for $IL\text{-}1_\alpha$ and $IL\text{-}1_\beta$ protein determination in our MØ supernates. The protein bands are scanned, using a Hoefer scanning densitometer, and the data are further analyzed by Hoefer densitometer software connected to our Compaq computer. As Fig. 1 illustrates, we demonstrated greater Il-1 protein amounts in the $FcRI^-$MØ supernates, than in the $FcRI^+$MØ supernates. This observation might give partial explanation to the better APC capacity seen in the $FcRI^-$ MØ subsets. In the future, we are planning to assess the possible differential $IL\text{-}1_\alpha$ and $IL\text{-}1_\beta$ production in the immunocompromised post-injury patients' MØ using Western-blotting techniques.

We made significant progress this year in testing TNF production by post-injury patients' MØ. TNF_α, also called Cachetin, is implicated in mediating septic shock and reportedly is elevated in the blood of some septic patients. Therefore, we examined the TNF production of patients and normals MØ as well as the MØ subsets after different stimulation. MØ from 11 trauma and 11 burn patients were suboptimally stimulated with interferon$_\gamma$ (IFN_γ) plus the synthetic bacterial cell wall analogue, muramyl dipeptide (MDP). TNF secretion by the patients' MØ was significantly increased compared to the normals. Nevertheless, stimulated patients' MØ did not always show secreted TNF levels above the range of the maximally stimulated normals (Fig.2). However, where total MØ TNF (cell-associated plus secreted TNF) was assessed, we found a tremendous elevation in the TNF levels of the immunocompromised trauma patients'

MØ. Fig. 3 illustrates the three typical patterns for the TNF production in our post-injury patients where MØ were stimulated with IFN+MDP. In one group of patients (6 out of 14) low initial TNF levels were observed at days 1-4 post-injury, with high preseptic TNF levels after day 5 post-injury. Most of the TNF elevation was cell-associated. A second group of patients (3 out of 14) experienced moderate initial MØ TNF levels, followed by moderate elevations in their total TNF presepsis. In the third group of patients, (5 out of 14) their initially assessed MØ TNF levels were already high on 1-4 days post-injury, and their MØ TNF levels further increased presepsis.

In summary, these suboptimally stimulated (IFN_γ + MDP) MØ TNF levels show that all immunocompromised trauma and burn patients experienced highly significant increases in their MØ TNF production during post-injury and preseptic periods. It can also be concluded, that although TNF secretion increases after injury in the patients' MØ, the cell-associated MØ TNF represents the majority of the pathologically elevated MØ TNF levels in these immunocompromised patients. When patients' MØ TNF levels were tested without in vitro stimulation, secreted MØ TNF levels were always negligible. As illustrated in Figure 4, however, immunocompromised post-injury patients MØ had significantly increased cell-associated TNF activity at preseptic periods even without in vitro stimulation, compared to their corresponding levels produced during nonseptic periods. ($P<0.001$). This observation strongly suggests that the post-trauma preseptic microenvironment is highly stimulatory for MØ. These findings might have applications in a forward area to select out the patients at high risk for septic complications before their clinical symptoms would occur. The elevation in MØ TNF levels (especially the cell-associated TNF) always preceded the actual septic episodes in our experiments. We have shown that elevated patients' MØ TNF levels correlate with their clinical course. The peak elevation in total MØ TNF levels of patients' MØ correlated with the onset of septic episodes and often occurred as early as 3 days prior to overt sepsis (Table 5). Patients who did not develop sepsis had total TNF levels well below the levels of most patients with septic complications. Patients (4 out of the total 22) had massive elevated total TNF (>180 ng $/10^6$ MØ) during day 1-3 post-injury and had 100% mortality. Observation of high cell-associated MØ TNF activity in the preseptic immunocompromised patients might also have an impact on their therapeutic manipulation. Our experiments using the cyclo-oxygenase inhibitor, Indomethacin, demonstrated that Indomethacin has a dual effect on preactivated patients' MØ. First, Indomethacin increases the total TNF production in both normals' and patients' MØ. Second, Indomethacin can redistribute the already high levels of cell-associated MØ TNF to the secreted form in the immunocompromised patients. Consequently, cyclo-oxygenase inhibitor treatment in a post-injury patient, who already has high cell-associated MØ TNF levels, could easily induce an increase in their MØ TNF secretion with subsequent septic shock. Cyclo-oxygenase inhibitor treatment on the other hand could be beneficial for downregulation of the abnormally elevated MØ PGE_2 levels in immunocompromised trauma and burn patients. Inhibitory effects of high PGE_2 levels on both T-cell and MØ functions have been repeatedly demonstrated. High MØ levels of PGE_2 have recently been shown to downregulate LPS induced MØ TNF production at the messenger RNA level. Therefore, post-trauma elevation of MØ TNF_α seems somewhat of an anomaly since increased MØ production of PGE_2 is also

documented as one of the aberrant MØ functions. Consequently, we followed the post-injury patients' MØ PGE_2 and TNF production simultaneously. Figure 5 illustrates corresponding unstimulated MØ PGE_2 and TNFdata for 3 patients, to demonstrate that high MØ TNF levels were produced in the face of high PGE_2 levels (20-50 mg/ml) in these post-injury patients' MØ. Patients' MØ concomitantly increased their TNF and PGE_2 production upon IFN_γ + MDP stimulation (Fig. 6). Although high PGE_2 levels downregulate the TNF at the mRNA levels in normal MØ, this effect did not appear in the immunocompromised post-injury patients' MØ. TNF was clearly synthesized by the patients' MØ in the face of high MØ PGE_2 levels (Fig. 7).

In summary, during this contract period, we have demonstrated that immunocompromised post-injury patients' MØ have massively elevated cell-associated TNF levels with a subsequent increase in MØ TNF secretion. Elevation in MØ TNF levels appears 2-3 days prior to sepsis and patients with extremely high total TNF levels (>180 ng/10^6 MØ) during day 1-3 post-injury have almost 100% mortality. These results suggest that testing MØ TNF levels can help to select post-injury patients who need more intensive care and therapy. As part of our exploration of prophylactic modalities, we have examined human recombinant interleukin 4 (IL-4) on post-injury patients' MO functions. We demonstrated a novel inhibitory effect of IL-4 on MØ PGE_2 and MØ TNF in normals' and patients' MØ. IL-4 downregulated MØ PGE_2 levels unstimulated, as well as after MDP stimulation (Fig. 8). IL-4 could downregulate MØ PGE_2 production by MDP stimulated MØ even in the high constituitively PGE_2 producing $FcRI^+$ MØ subset of immunosuppressed trauma patients. (Fig. 9). IL-4 also downregulated normals' and patients' MØ TNF production (Fig. 10). These imply that IL-4 might have beneficial effects on the aberrant MØ functions in immunocompromised trauma and burn patients.

We made progress this year in more complete monitoring of post-trauma patients' MØ functions of post-injury patients developing a bioassay for determination of Interleukin 6 (IL-6). IL-6 is produced by different cell types, including MØ and has a major role in T-cell activation. Elevated serum IL-6 levels were reported in post-injury patients. Therefore, we examined the IL-6 production of patients and normals' MØ and MØ subsets after different stimuli. As shown in Table 6, both normals' and patients' MØ produce IL-6 without any additional stimulation other than the MØ separation procedure itself. This basal MØ IL-6 production, however, increases upon stimulation with MDP both in the normals and in the patients. We found stimulated MØ IL-6 production to be greater in the patients' MØ compared to the normal controls. Therefore, the MØ might be the actual source of the previously reported elevated IL-6 in post-injury patients' sera. Testing IL-6 production by the patients' and normals' FcRI MØ subsets, we observed a greater IL-6 production by the $FcRI^+$ MØ subset (Table 7). Since the $FcRI^+$ MO represents a main IL-6 producing subset, the greater IL-6 levels of the trauma patients' MØ could be explained by the increased proportion of the $FcRI^+$ MØ subset in these post-injury patients. As we have previously shown, the immunocompromised patients experience a shift in their $FcRI^+/FcRI^-$ MØ ratio towards the $FcRI^+$ MØ. This FcRI MØ ratio imbalance can be correlated with several MØ aberrations detected in the post-injury patients. The $FcRI^+$ MØ subset produces the majority of MØ PGE_2, TNF, as well as

IL-6, and increased levels of these monokines have been reported post-injury. Therefore the observation of an increase in the $FcRI^+$ MØ subset ratio in immunocompromised post-injury patients could be a predictor of a poor prognosis.

During this contract year, we have also initiated the mv1Lu bioassay for transforming growth factor beta (TGF_β) determination. TGF_β has several inhibitory effects on the immune functions. Since most of the impaired immune functions after injury can not be be solely explained as resulting from the inhibiting effect of high MØ PGE_2 levels, we assume that other inhibitory mediators might be involved. For example, post-injury MØ might produce increased levels of the immunosuppressive TGF_β. Our preliminary data suggest, that immunocompromised trauma and burn patients' MØ do produce greater TGF_β activity than the normals' MØ. These results need to be confirmed during the next contract period. We are also seeking any correlation between MØ TGF_β levels and clinical outcome on prognosis in the post-trauma and burn patients. This contract year has been marked by a great deal of progress in initiating new bioassays for monitoring patients' immunofunction, defining the cellular basis for the immunoaberrations seen in immunocompromised patients, and in investigating possible prophylactic regimens. The following papers have been produced during this contract period:

1. Szabo G, Kodys K, Miller C: 1989. $FcRI^+$ Monocyte (MØ) Subset Produces the Majority of MØ IL-6. 7th International Congress of Immunology, Berlin, West Germany, in press.

2. Takayama T, Miller-Graziano C, Szabo G, et al: 1989. Elevated Tumor Necrosis Factor (TNF) Production Concomitant to Elevated PGE_2 by Trauma Patients; Monocytes (MØ). Archives of Surgery, in press.

3. Miller C, Szabo G, Takayama T: 1988. *Interleukin-4 (IL-4(Effects on* Monocyte PGE_2 and Tumor Necrosis Factor (TNF). Journal of Leukocyte Biology 44:273.

4. Szabo G, Miller C, Wu J, Kodys K: 1988. Characteristics of Human Cd4 Monocyte (MØ) Subsets. Journal of Leukocyte Biology 44:276.

5. Miller-Graziano C, Szabo G, Takayama T, Wu J: 1989. Alterations of Monocyte Function Following Major Surgery. In (Faist, E. Ed.) The Immune consequences of Trauma, Shock and Sepsis. Karl Demeter Verlag, in press, pp. 9-16.

6. Miller-Graziano C, Find M, Wu J, Szabo G, Kodys K: 1988. Mechanisms of Altered Monocyte Prostaglandin E_2 Production in Sevrely Injured Patients. Arch. Surgery 123:293.

7. Miller-Graziano C, et al: 1989. Systemic Consequences of Intestinal Ischemia: Immunological Disturbances. In (Fiddian-Green, R., Ed.) Splanchnic Perfusion and Multiple Organ Failure. Edward Arnold Co., Inc., Baltimore, pp. 237-247.

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1. Kramer and Carver, 1986, J. Immun., 93:201.
2. Van Oers, M.H.J., van der Heyden, A.A.P.A.M., Aarden, L.A. (1988) Clin. Exp. Immunol. 71 , 314.

Table 1. Correlation of MØ PA Depression, PGE_2 Augmentation, and Clinical Outcome

Patient	Injury	PHA[a]	PA[b]	PGE_2[c]	Outcome
1. Ma	Truama	-95	19.2→11.4	2.0→15.0	Fatal Sepsis
2. Do	Trauma	-71	22.4→15.3	4.6→108.0	Staph Pneumonia
3. Sn	Trauma	-42	18.1→16.8	95.0(9.3)	E. Coli Pneumonia
4. Mu	Trauma	-39	No data[d]	21.8	Fatal Sepsis
5. Cl	Trauma	-69	16.9(25.1)	2.0→15.0	Fatal Sepsis
6. Mo	Trauma	No Data	10.4(22.7)	No Data	Fatal Sepsis
7. Go	Trauma	-64	9.3→0.2	54.5(28.8)	Enterobacter Sepsis
8. Ha	Trauma	-84	11.5→9.3	63.2→102.4	Strep Pneumonia
9. Fl	Trauma	-83	16.5(26.1)	1.1→18.9	Fatal Sepsis
10. Gr	Burn	-74	5.1(25.2)	4.7→51.2	Multiple Sepsis
11. Fl	Burn	-83	3.03(24.2)	No Data	Fatal Sepsis
12. Ro	Burn	-65	20.9→11.7	3.1→37.0	Febrile Episodes
13. Tr	Burn	-46	13.2→1.8	32.0(5.6)	Wound Infection
14. Th	Burn	-76	15.1(22.61)	101.0(78.0)	Septic Episode
15. Mc	Burn	-87	28.7→0.9	5.8→65.0	Multiple Sepsis
16. Na	Burn	-81	30.9→15.9	10.0→38.0	Multiple Sepsis
17. Ly	Burn	-50	21.9→15.2	4.7→25.0	Febrile Episodes
18. Mg	Burn	-92	27.7→12.6	5.9→99.0	Staph Pneumonia
19. Be	Burn	-85	9.26(24.2)	11.9→34.7	Multiple Sepsis
20. Ge	Burn	-88	15.1(20.5)	5.9→14.0	Febrile Episodes
21. Ng	Burn	-68	18.3→13.1	4.2→25.8	Fatal Sepsis
22. Un	Burn	ND	19.4→6.3	77.1(29.3)	Staph Sepsis

a) Maximum percent change in phytohemagglutinin (PHA, 2mg/ml) mitogen induced proliferation of $2x10^5$ peripherial blood mononuclear cells from post-injury patients at 2-12 days post-injury compared to the normals.

b) Maximum change in MØ plasminogen activator (PA) activity from initial response to most depressed during the first 13 days post-injury. PA activity was measured in % plasmin specific fibrinolysis. PA levels of the corresponding normal controls are shown in parenthesis, where only one patient MØ PA level was available.

c) Maximum changes in MØ Prostaglaudin E_2 (PGE_2) production within the first 13 days post-injury, PGE_2 measured as nanogram per 10^6 recovered MØ. PGE_2 of normal MØ controls are shown in parenthesis where only one patient PGE_2 level was tested.

d) Some patients were only assessed in subset experiments for TNF or IL-6 and PGE_2 data is derived from these experiments.

Table 2: Depressed APC capacity of pt MØ
(^{3}H-TdP cpm)

	Normal	Immunosuppressed Patient
$FcRI^+$	1,764	1,335
$FcRI^+p$[1]	1,769	857
$FcRI^-$	5,406	1,191
$FcRI^-p$	14,618	2,378

1. MØ pulsed(p) 24 hrs with Tetanus Toxoid

Table 3: Inability of $FcRI^+$MØ to act as APC isn't primarily due to excessive PGE_2

MØ Treat	Exp40	Exp37
$FcRI^+p$[1]	9,165	10,437
$FcRI^+p$+Indo	9,213	5,625
$FcRI^-p$	52,637	35,824
$FcRI^-p$+Indo	55,220	32,152

1. MØ pulsed(p) 24 hrs with Tetanus Toxoid

Table 4: Decreased Il-1 production in Immunosuppressed patients is expressed only in the $FcRI^+$MØ

Post-inj. Day[1]	Pt.	Norm
2 MØ[2]	11.8[3]	8.5
5 MØ	5.5	21.7
5 MØ	20.9	36.9
5 $FcRI^+$	16.4	44.9
5 $FcRI^-$	31.4	33.1

1. Day post-injury of MØ isolation
2. Unseparated MØ population or separated MØ subsets $FcRI^+$ (rosetting with antibody coated human erythrocytes) $FcRI^-$ (non-rosetting)
3. Stimulation index = proliferation of D10G4 T-cells to stimulated MØ unstimulated MØ

Table 5 Correlation of Patient MØ TNF_α (Unstimulated)[1] and Clinical Course

Pt. (age)	Injury	Total TNF_α[2]	Clinical Correlation
1. (21)	Trauma	33.28	S.Aureus Pneumonia Sepsis
2. (21)	Burn	39.80	Multiple Sepsis
3. (45)	Trauma	43.31	E.Coli Pneumonia Sepsis
4. (25)	Trauma	47.51	Multiple Sepsis
5. (47)	Trauma	53.85	Candida Sepsis
6. (31)	Trauma	57.98	Enterobacter Sepsis
7. (24)	Burn	64.05	E.Cloacae Wound Sepsis
8. (35)	Burn	70.35	S.Aureus Pneumonia Sepsis
9. (39)	Trauma	83.65	Multiple Sepsis
10. (32)	Trauma	121.03	Grp D Strep Sepsis
11. (48)	Burn	132.28	Multiple Sepsis
12. (54)	Burn	134.09	Fatal Sepsis
13. (47)	Burn	156.97	S.Aureus Wound Sepsis
14. (37)	Burn	164.03	Candida Sepsis
15. (21)	Burn	175.69	Wound Sepsis
16. (37)	Trauma	187.59	Multiple Sepsis (fatal)
17. (83)	Burn	969.20	Multiple Sepsis (fatal)
18. (21)	Trauma	2.30[3]	No Complications
19. (23)	Trauma	8.81[3]	Febrile episode
20. (34)	Burn	18.18[3]	Febrile episode
21. (30)	Trauma	27.56[3]	Febrile episode
22. (61)	Burn	29.66[3]	Febrile episode

Normal	Unstimulated	0-2.28 (range),	Median=0
Normal	Stimulated[4]	0-18.20 (range),	Median=2.91

1. Unstimulated = no <u>in vitro</u> stimulation of isolated MØ.
2. Total TNF_α (ng/10^6 MØ/ml) during septic episodes, (post-injury day 3 - 30).
3. Maximum total MØ TNF_α of patients without septic episodes.
4. Stimulation = 20U/ml IFN_γ + 20μg/ml MDP

Table 6: Increased IL-6 Production by Patients MØ (U/ml

Exp. No.		Pt.	Norm
Exp. 205	Unstimulated	241.0	59.5
	MDP	208.0	80.5
Exp. 202	Unstimulated	53.5	75
	MDP	339.0	97.5
Exp. 188	Unstimulated	568.0	200
	MDP	780.0	590

Table 7: Greater IL-6 Production by the $FcRI^+$ MØ subset (U/ml)

	Exp. #205		Exp.# 202		Exp.# 188	
	Pt.	Norm	Pt.	Norm	Pt.	Norm
$FcRI^+$	366	136	472	212	1,553	721
$FcRI^-$	164	12	52	26	710	31

	Exp.# 201	Exp. #198
$FcRI^+$	620	312
$FcRI^-$	255	60

Table 8: Increased TGF_β Activity in the Patients' MØ Supernates (pM/ml)

Exp. #2		Exp. #3		Exp.#4			
Pt. C	Norm	Pt. McC	Norm	Pt. M	Norm	Pt. G	Norm
5.3	0	4.3	0	337	0	291	0

Exp. #5		Exp. #6		Exp. #12			
Pt. B	Norm	Pt. M	Norm	Pt. G	Pt. McC	Pt. N	Norm
114	0	5.6	0	165	77	193	0

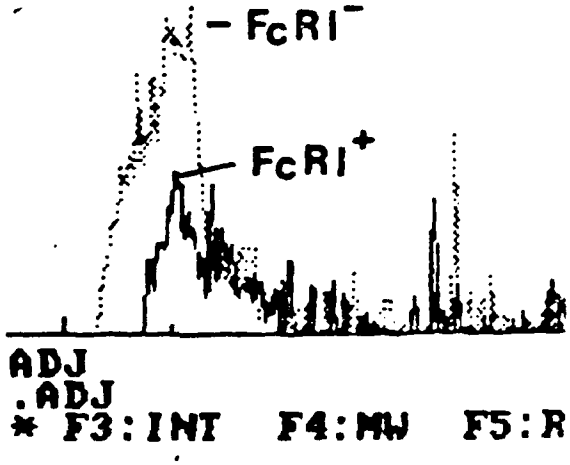


Fig. 1. Differential production of IL-1 by $FcRI^+$ versus $FcRI^-$ MØ as detected by isoelectro focusing.

Fig. 2A.

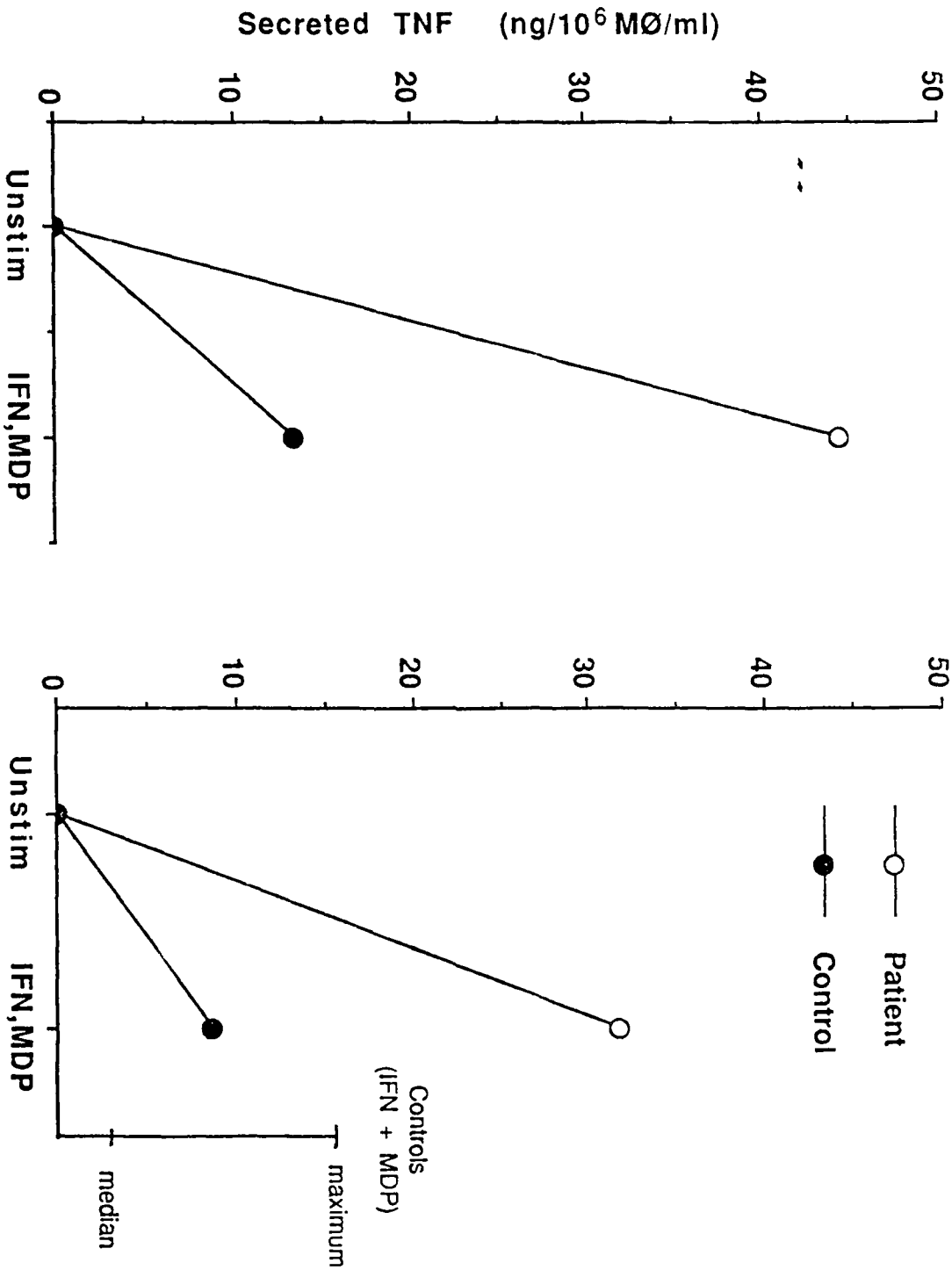

Secreted TNF (ng/10⁶ MØ/ml)
0
10
20
30
40
50
Unstim
IFN,MDP
Patient
Control
Controls
(IFN + MDP)
maximum
median

Fig. 2B.

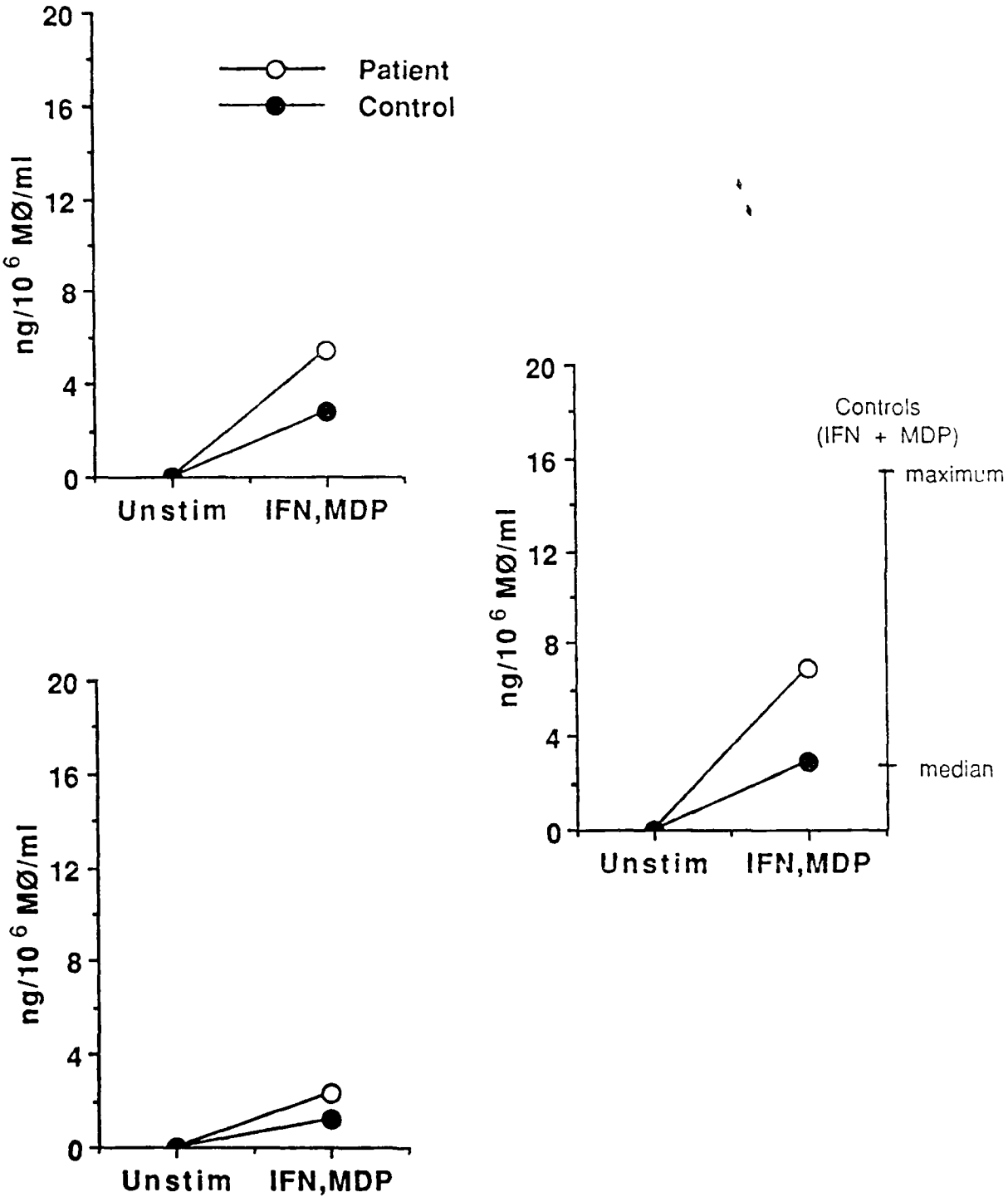

20
16
12
8
4
0
ng/10 6 MØ/ml
Patient
Control
Unstim
IFN,MDP
Controls
(IFN + MDP)
maximum
median

Fig. 3A.

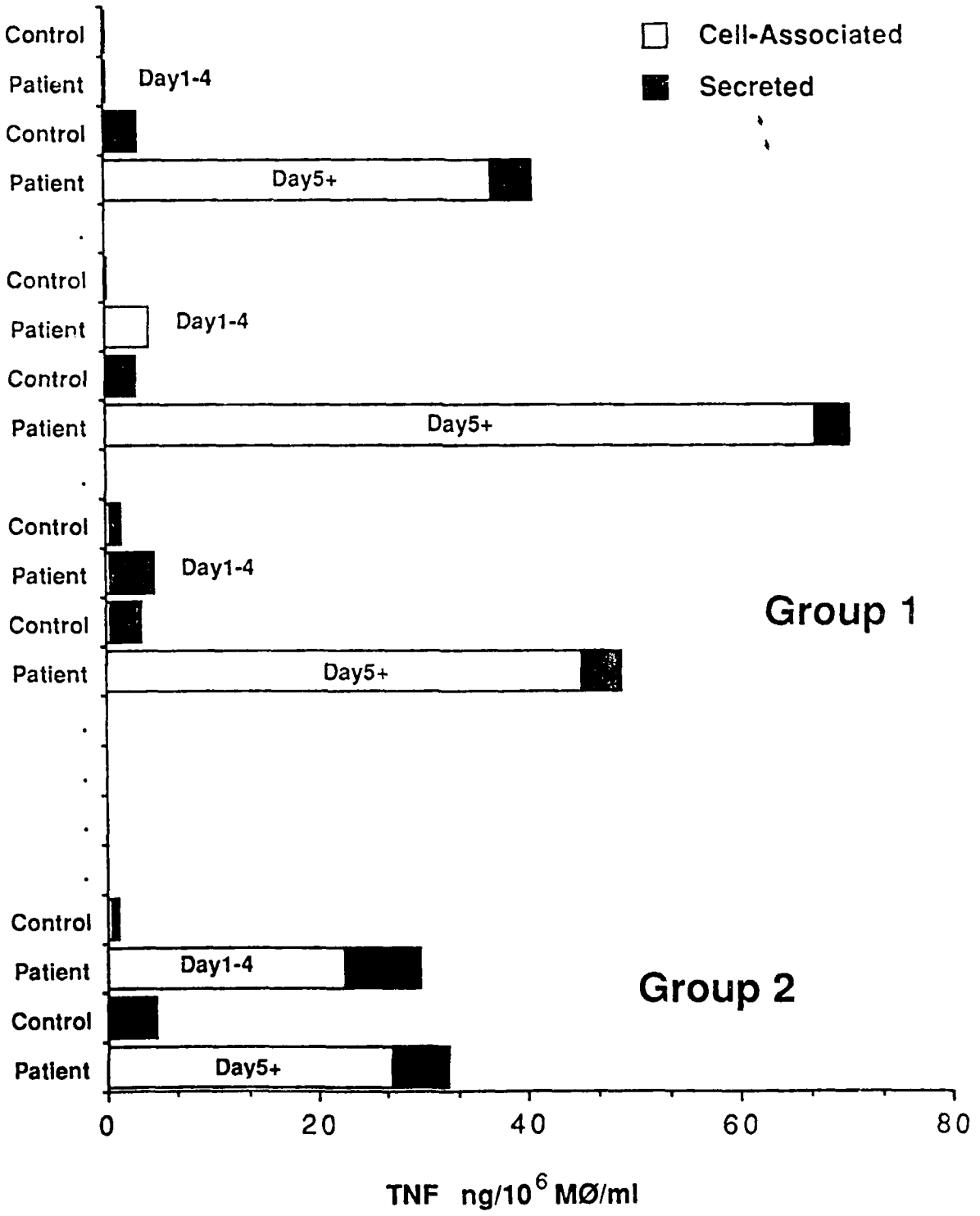

Control
Patient
Day1-4
Control
Patient
Day5+
Control
Patient
Day1-4
Control
Patient
Day5+
Control
Patient
Day1-4
Control
Patient
Day5+
Control
Patient
Day1-4
Control
Patient
Day5+
Cell-Associated
Secreted
Group 1
Group 2
0
20
40
60
80
TNF ng/10^6 MØ/ml

Fig. 3B.

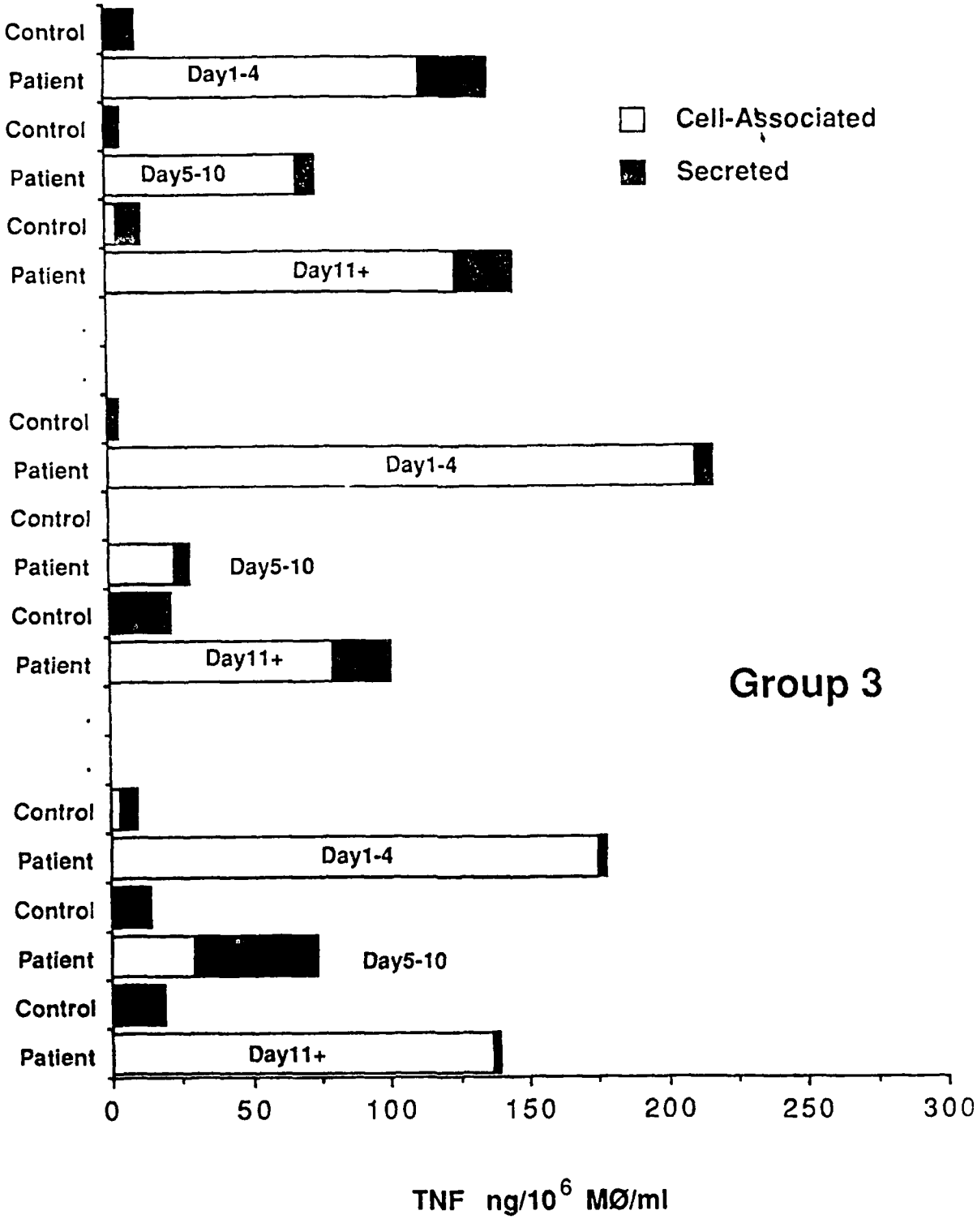
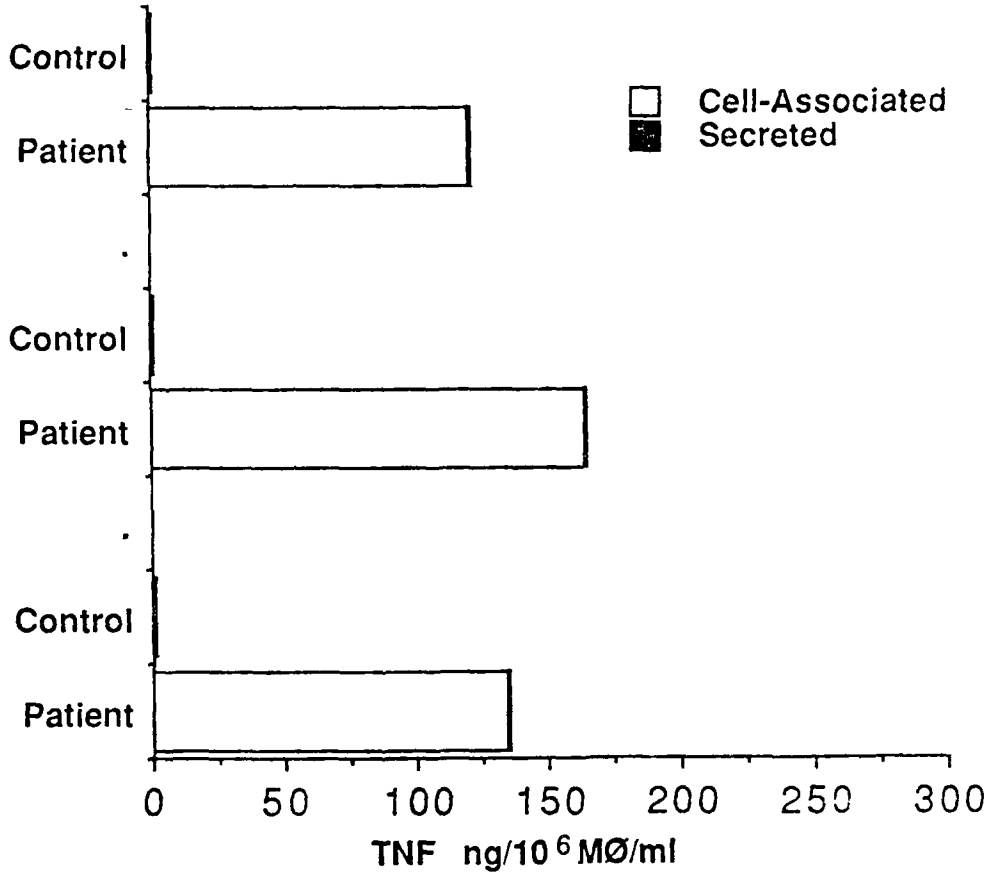

Control
Patient
Day1-4
Control
Patient
Day5-10
Control
Patient
Day11+
Cell-Associated
Secreted
Control
Patient
Day1-4
Control
Patient
Day5-10
Control
Patient
Day11+
Group 3
Control
Patient
Day1-4
Control
Patient
Day5-10
Control
Patient
Day11+
0
50
100
150
200
250
300
TNF ng/10^6 MØ/ml

Fig. 4.


Control
Patient
Control
Patient
Control
Patient
Cell-Associated
Secreted
0
50
100
150
200
250
300
TNF ng/10 6 MØ/ml

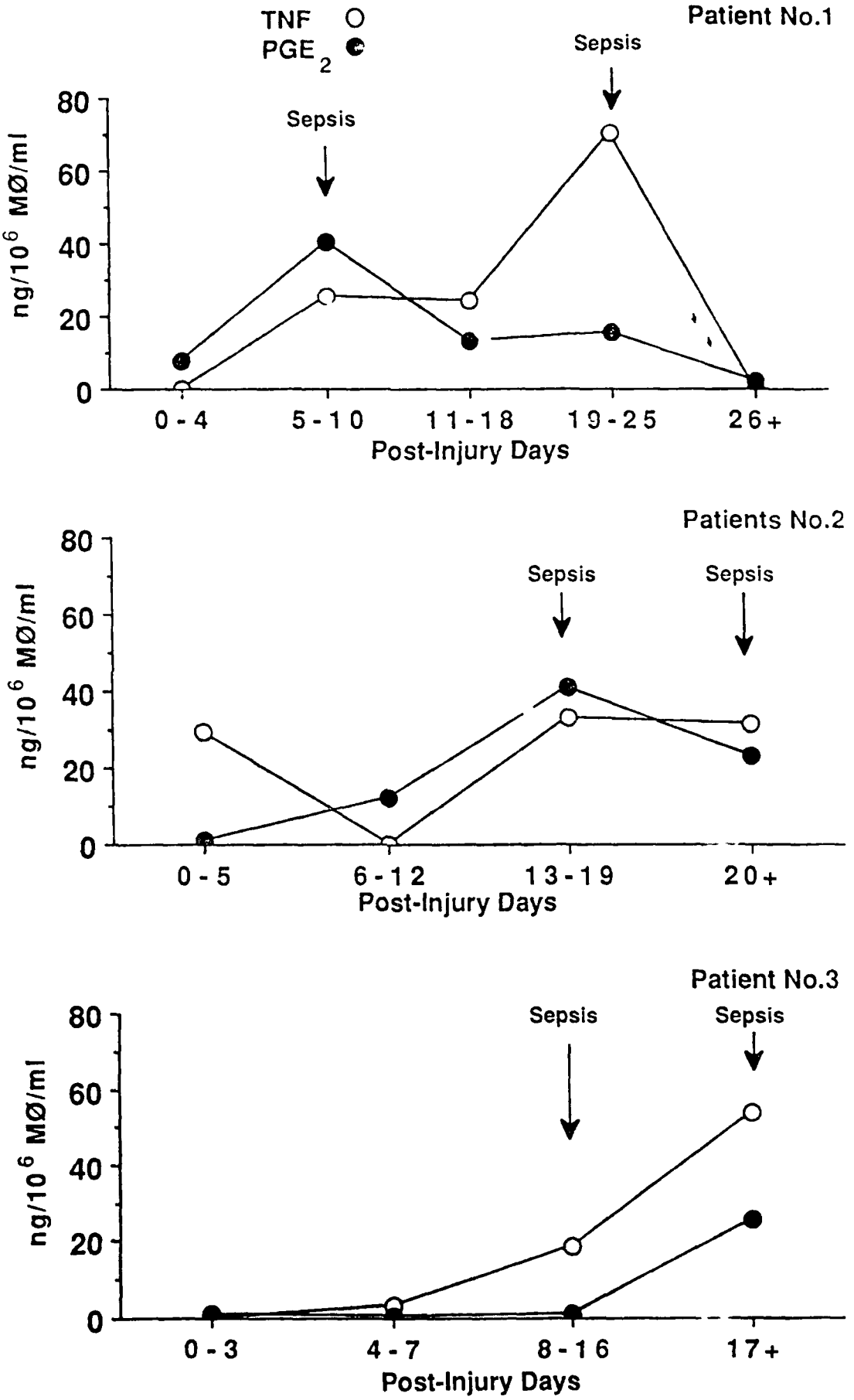

TNF
PGE2
Patient No.1
Sepsis
Sepsis
80
60
40
20
0
ng/10^6 MØ/ml
0-4
5-10
11-18
19-25
26+
Post-Injury Days
Patients No.2
Sepsis
Sepsis
80
60
40
20
0
ng/10^6 MØ/ml
0-5
6-12
13-19
20+
Post-Injury Days
Patient No.3
Sepsis
Sepsis
80
60
40
20
0
ng/10^6 MØ/ml
0-3
4-7
8-16
17+
Post-Injury Days

Fig. 6

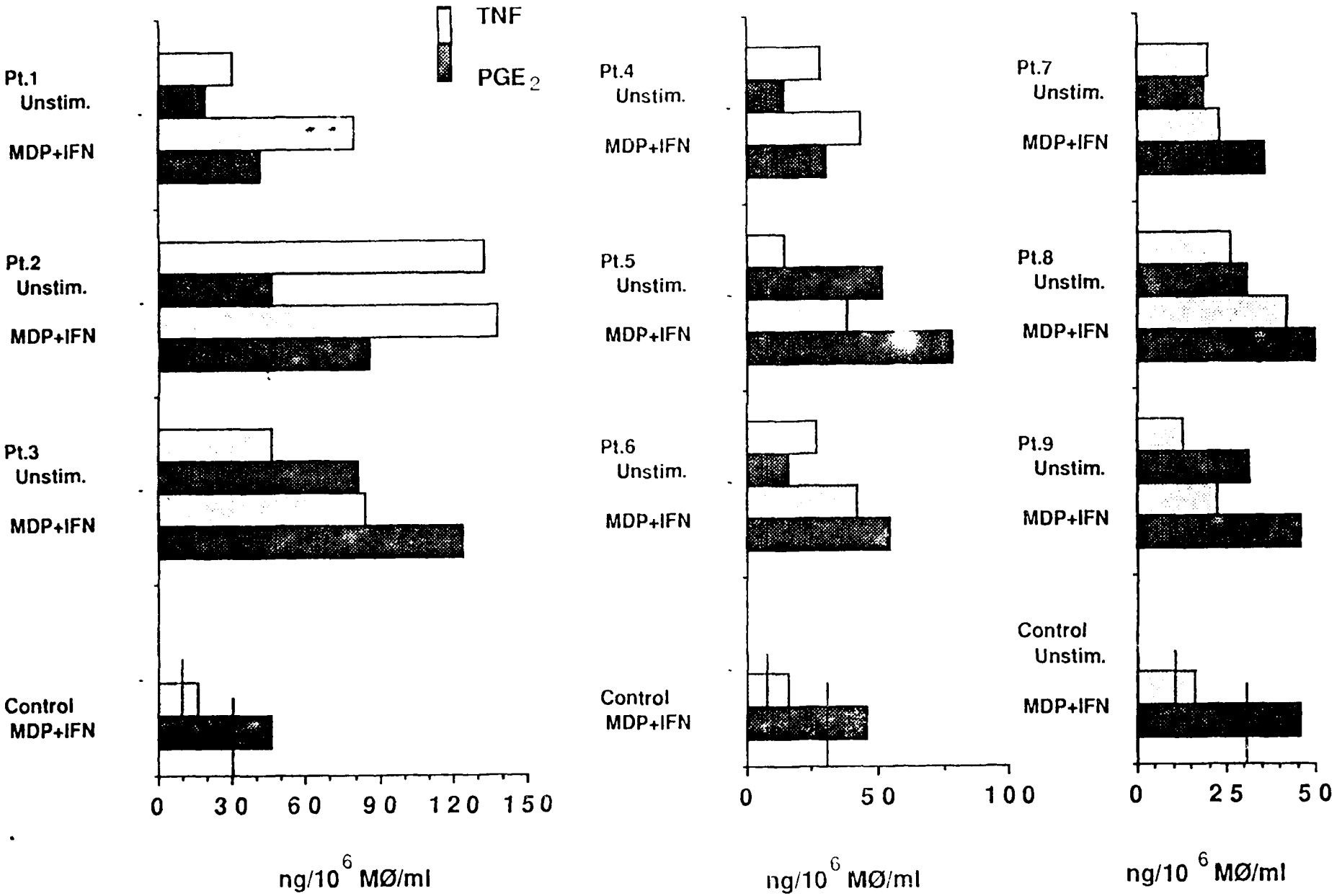

TNF
PGE2
Pt.1
Unstim.
MDP+IFN
Pt.2
Unstim.
MDP+IFN
Pt.3
Unstim.
MDP+IFN
Control
MDP+IFN
0
30
60
90
120
150
ng/10 6 MØ/ml
Pt.4
Unstim.
MDP+IFN
Pt.5
Unstim.
MDP+IFN
Pt.6
Unstim.
MDP+IFN
Control
MDP+IFN
0
50
100
ng/10 6 MØ/ml
Pt.7
Unstim.
MDP+IFN
Pt.8
Unstim.
MDP+IFN
Pt.9
Unstim.
MDP+IFN
Control
Unstim.
MDP+IFN
0
25
50
ng/10 6 MØ/ml

Fig. 7

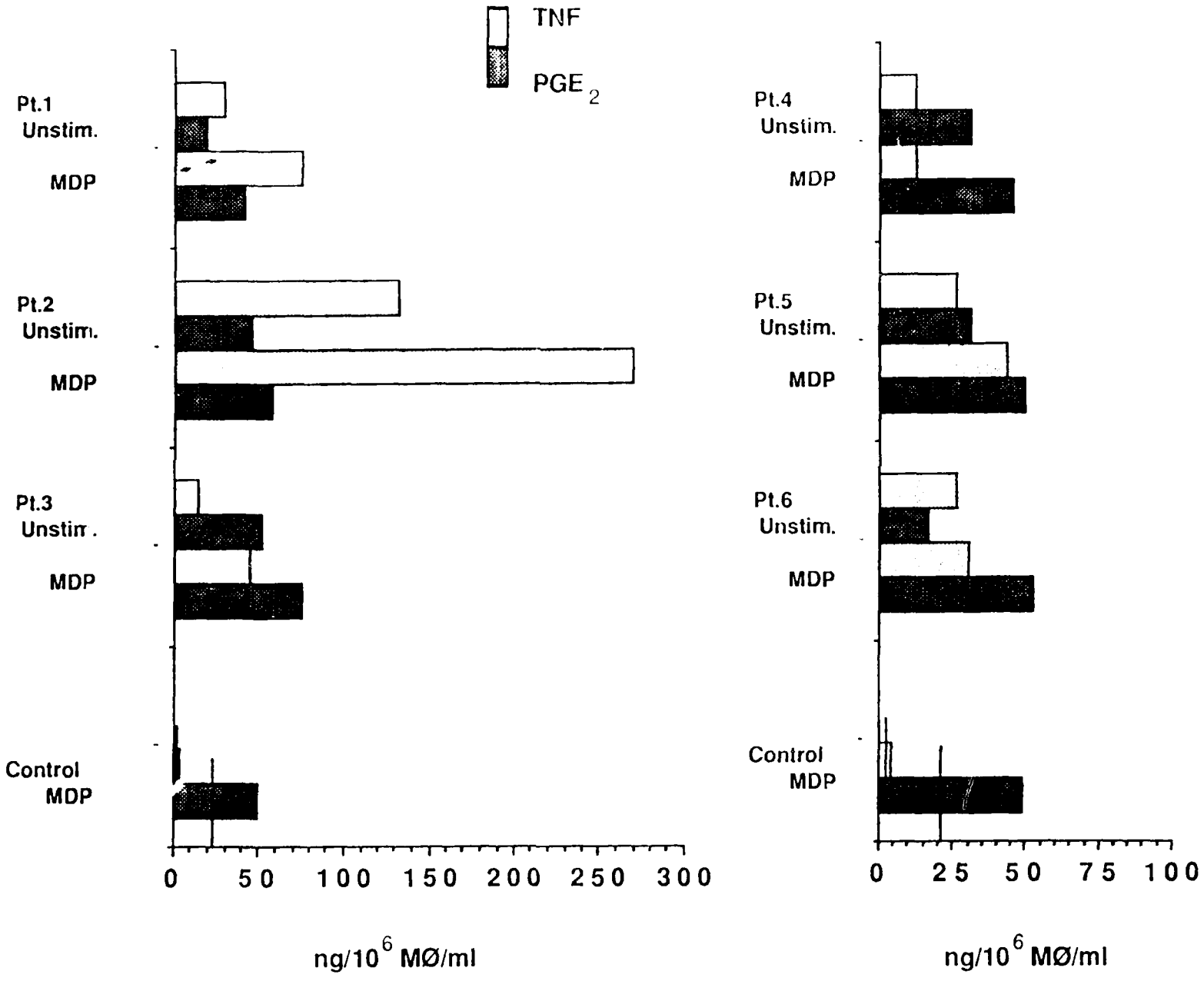

TNF
PGE2
Pt.1
Unstim.
MDP
Pt.2
Unstim.
MDP
Pt.3
Unstim.
MDP
Control
MDP
0
50
100
150
200
250
300
ng/10^6 MØ/ml
Pt.4
Unstim.
MDP
Pt.5
Unstim.
MDP
Pt.6
Unstim.
MDP
Control
MDP
0
25
50
75
100
ng/10^6 MØ/ml

Fig. 8

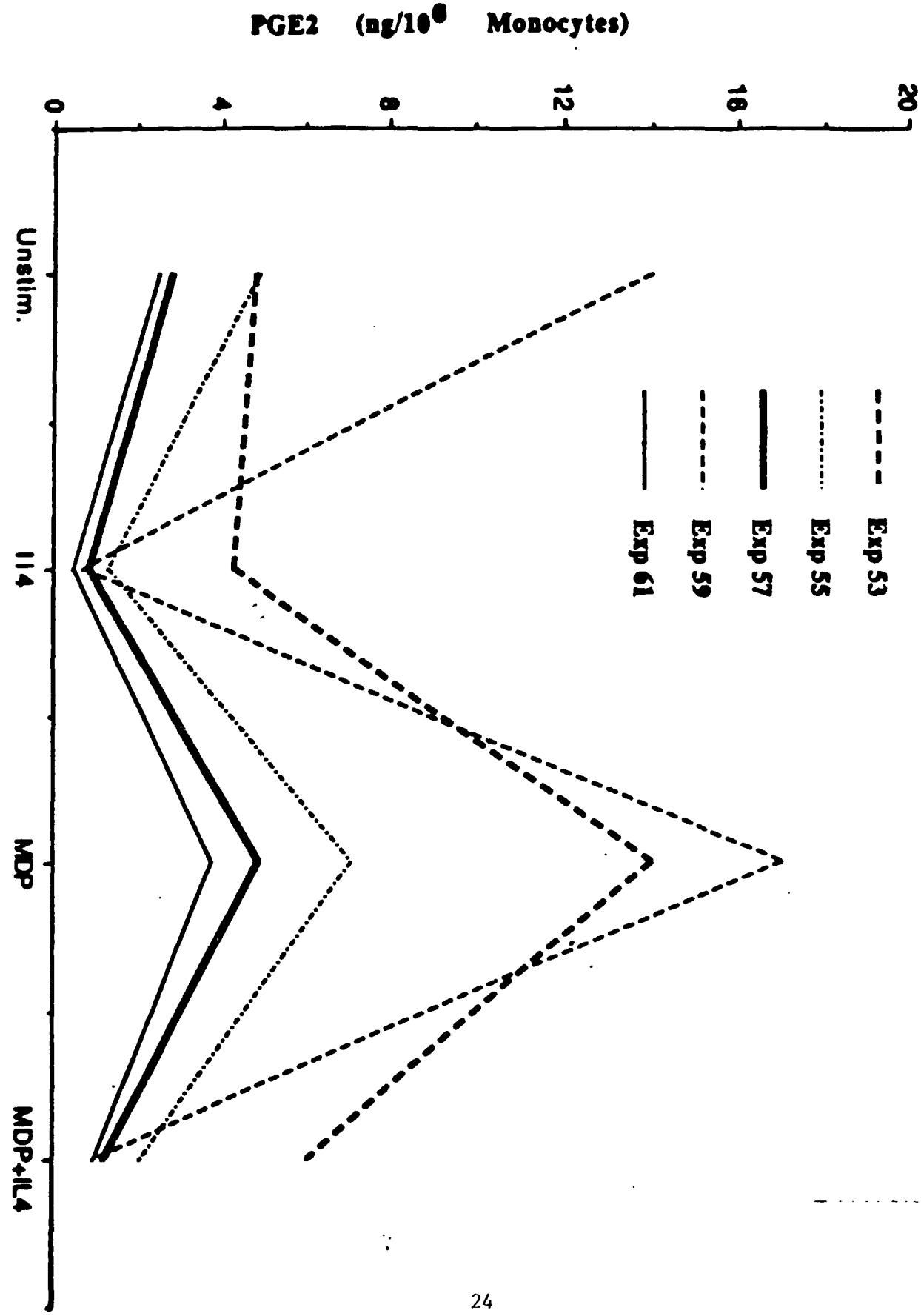

PGE2 (ng/10⁶ Monocytes)
0
4
8
12
16
20
Unstim.
IL4
MDP
MDP+IL4
Exp 61
Exp 59
Exp 57
Exp 55
Exp 53

Fig. 9

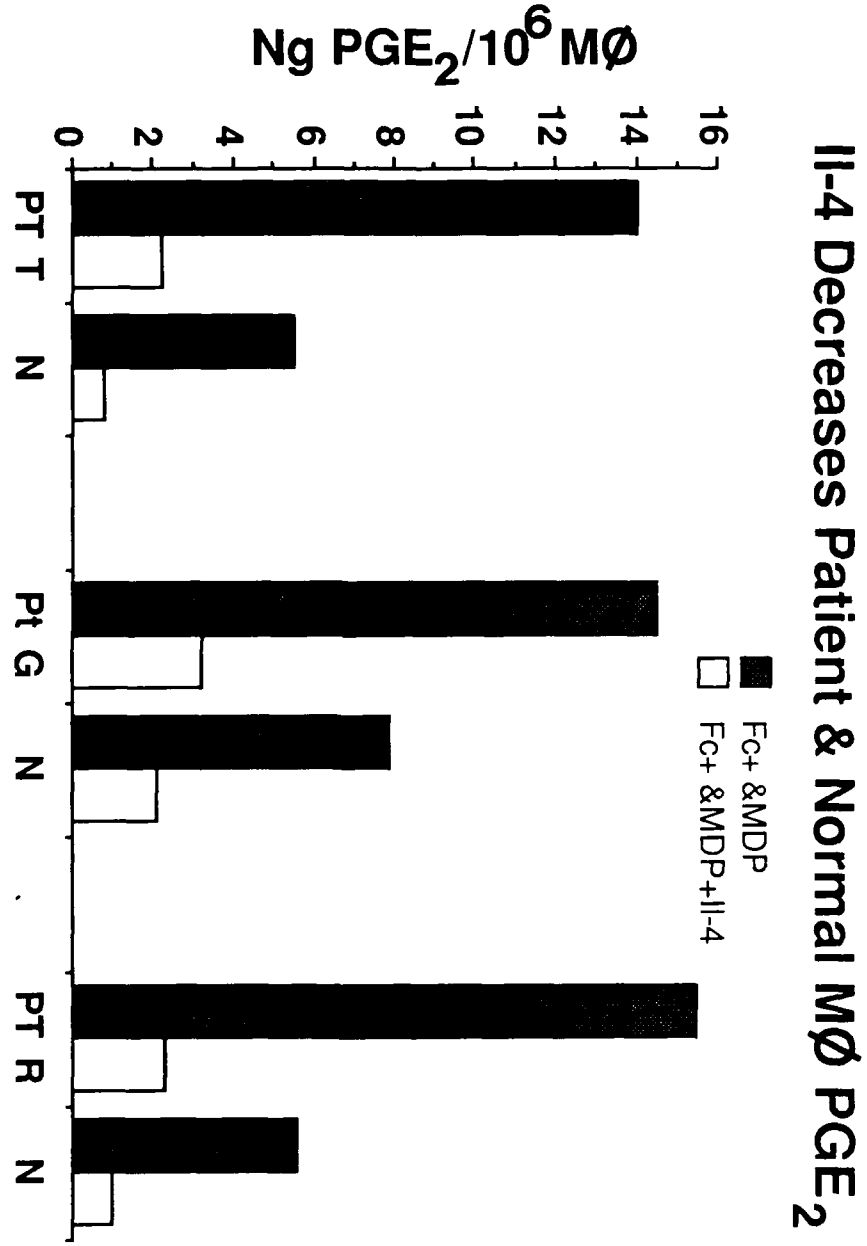
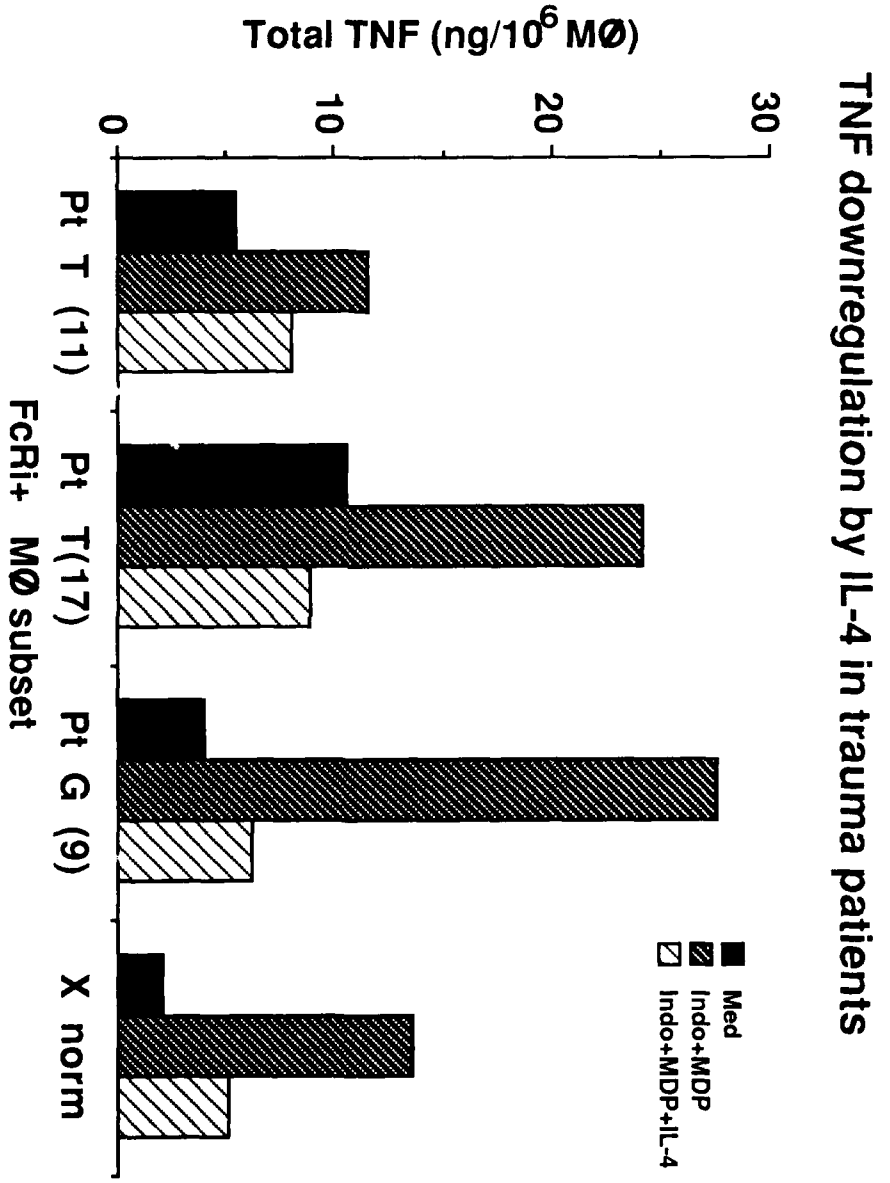

Il-4 Decreases Patient & Normal MØ PGE2
Ng PGE2/10^6 MØ
0
2
4
6
8
10
12
14
16
PT T
N
Pt G
N
PT R
N
Fc+ &MDP
Fc+ &MDP+Il-4

Fig. 10


TNF downregulation by IL-4 in trauma patients
Total TNF (ng/10^6 MØ)
0
10
20
30
Med
Indo+MDP
Indo+MDP+IL-4
Pt T (11)
Pt T(17)
Pt G (9)
X norm
FcRi+ MØ subset

LEGENDS

Fig 2A. Significantly increased secreted TNF_α of septic patients' and controls' MØ after stimulation (10-20U/ml IFN_γ + 20μg/ml MDP); 2 representative patients out of 5 with greatly elevated TNF_α levels. Maximum and median TNF_α levels of stimulated MØ controls (far right axis).

Fig 2B. Minimally increased secreted TNF_α of septic patients' and controls' MØ after stimulation (IFN_γ + MDP); 3 representative patients of 12 with TNF_α levels near or within normal range; maximum and minimum TNF_α levels of all controls' stimulated MØ (far right axis).

Fig 3A. Stimulated MØ cell-associated (open bars) and secreted (filled bars) TNF_α levels of Group 1 (low initial, high preseptic TNF_α; 6/14) and Group 2 (moderate initial, moderate preseptic TNF_α; 3/14) representative septic patients as well as controls at several post-injury days.

Fig 3B. Stimulated MØ cell-associated (open bars) and secreted (filled bars) TNF_α levels in representative patients of Group 3 (high initial, high preseptic TNF_α; 5/14) septic patients and controls at several post-injury time periods (indicated as post-injury day).

Fig 4. Pre-existing elevated levels of cell-associated (open bars) but not secreted (filled bars) MØ TNF_α in 3 representative out of 17 septic patients without <u>in vitro</u> stimulation. Cell-associated TNF_α levels in normal, unstimulated MØ equal zero.

Fig 5. Comparison of time course of the rise in septic patients' (n=9) unstimulated MØ PGE_2 (filled circles) to unstimulated appearance of patients' MØ high levels of total TNF_α (cell-associated plus secreted MØ TNF_α; open circles). Normal unstimulated TNF_α levels equals 0-1ng/10^6 MØ/ml.

Fig [illegible]. Concomitantly elevated production of PGE_2 (dark-filled bars) and total TNF_α (cell-associated plus secreted TNF_α; light-filled bars) in monocytes from 9 septic trauma patients after stimulation (10-20U/ml IFN_γ plus 20μg/ml MDP). Control maximum (total bar) as well as median (crossline) are indicated.

Fig [illegible]. Concomitantly elevated production of MØ PGE_2 (dark-filled bars) and newly synthesized MØ total TNF_α (light-filled bars) after 20μg/ml MDP stimulation alone in 6/7 septic trauma patients. Control maximum (total bar) as well as median (crossline) are indicated.